

# Methods for Mass Rearing of *Aedes aegypti* (L.)

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LABORATORY rearing of *Aedes aegypti* (L.) has been a common procedure among entomologists and other scientists for about 50 years. Many improvements in equipment and techniques have been developed (1-3), and production by these methods has proved adequate for studies using as many as several thousand specimens. Experiments on dispersal (4) and on control by release of sexually sterile males (5, 6), however, require the sustained production of millions of specimens. This paper describes equipment and procedures developed during 1955 and 1956 for rearing broods of as many as 1.3 million larvae.

## Procedures

*Maintenance of adult colonies.* The strain of *A. aegypti* used in the studies had been maintained in the Technical Development Laboratories of the Communicable Disease Center in Savannah, Ga., since 1944. Two laboratory rooms, each 10 by 13 by 10 feet and maintained at about 80° F. and 80 percent relative humidity, were required for the mass production of *A. aegypti*. One room was used for adult colonies, emergence cages, and egg storage. The second room contained the larval rearing trays.

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Six mosquito colonies were used for egg production. Each colony consisted of approximately 10,000 adults and was maintained in a cage about 22 by 22 by 22 inches (fig. 1). A hinged platform within a screened tunnel through the cage was used to facilitate the feeding of the female mosquitoes upon the rabbit host (7). Each day for 4 hours the rabbit was placed in a metal tray 21 by 6 by 1.5 inches and inserted into the tunnel on the platform. The platform was wedged upward to force the clipped back of the rabbit against the 20-mesh screen of the tunnel. A wood block, 6 by 5.5 by 1.5 inches, placed beneath the rabbit's abdomen forced its back against the screen. The mosquitoes readily fed on the rabbit through the screen. The outer end of the tray, 3.5 inches high, served as a barrier to prevent the rabbit's escape. This cage eliminated the loss of mosquitoes that usually occurs while a host animal is being placed in or removed from a colony cage. Seedless raisins soaked in water, a cotton pad soaked with a 1-molar sucrose solution, and water were provided fresh each week as nourishment.

To maintain a relatively uniform population in each colony, approximately 3,000 pupae were added weekly. To prevent oviposition in the dish (6 inches in diameter and 3 inches high) containing the pupae, it was covered with a screen. The screen was removed briefly each day to permit the escape of the young adults. Each week, colony cages were cleaned and inspected for the presence of spiders or other predators.



**Table 1. Percent of *A. aegypti* hatch observed during 4-hour periods with eggs of known ages**

Age of eggs (weeks)	Number of trials <sup>1</sup>	Percent hatch	
		Range	Average
1-5-----	78	84-92	88
6-10-----	85	78-88	83
11-15-----	53	46-75	62
16-18-----	9	22-41	31

<sup>1</sup> Several hundred eggs per trial.

container was primarily dependent upon the consideration of laboratory space and ease of feeding, draining, and cleaning. A galvanized metal tray, 2 by 10 by 72 inches, was designed as the standard rearing container. Each tray had a 3/4-inch copper-tube outlet to facilitate draining and was stacked on a metal rack made of 1/8- by 3/4- by 3/4-inch angle iron. Eight racks held 24 rearing trays each for a total of 192 trays. Detailed specifications for construction of the trays and racks are given in figures 2 and 3.

To prevent any adverse reaction between the galvanized metal and the medium, each tray was coated on the inside with a thin layer of paraffin. This was readily accomplished by applying melted paraffin to preheated trays. After

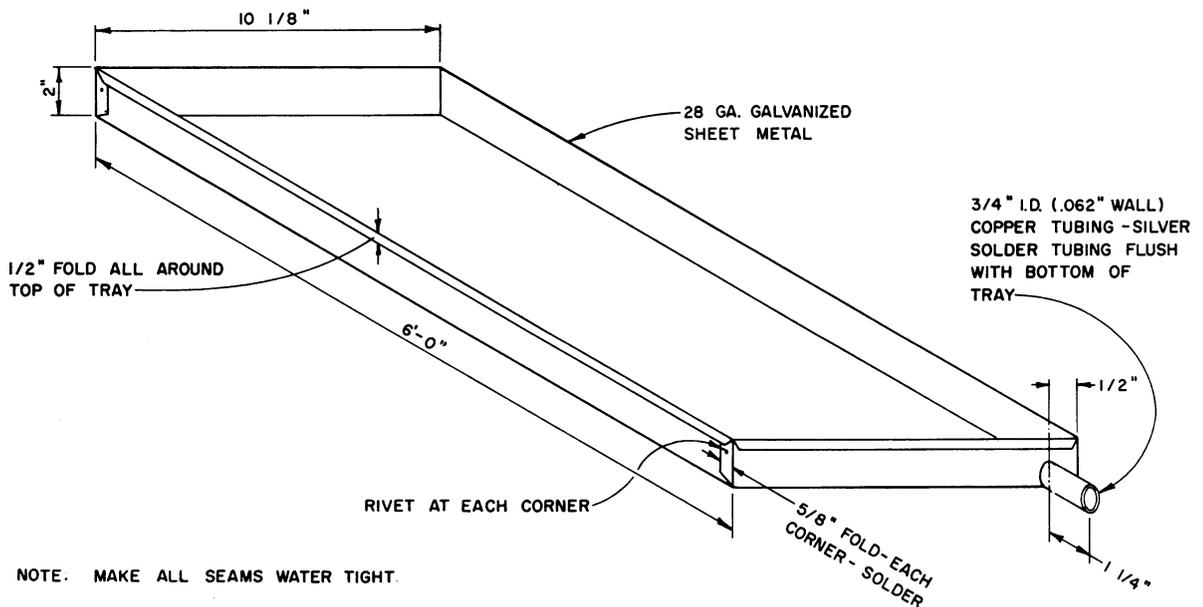
the tray was lightly coated, the excess wax was poured into the next tray.

*Estimation of larval numbers.* A total of 7,000 larvae were reared in 7 liters of water in each tray. Water loss by evaporation was replenished daily.

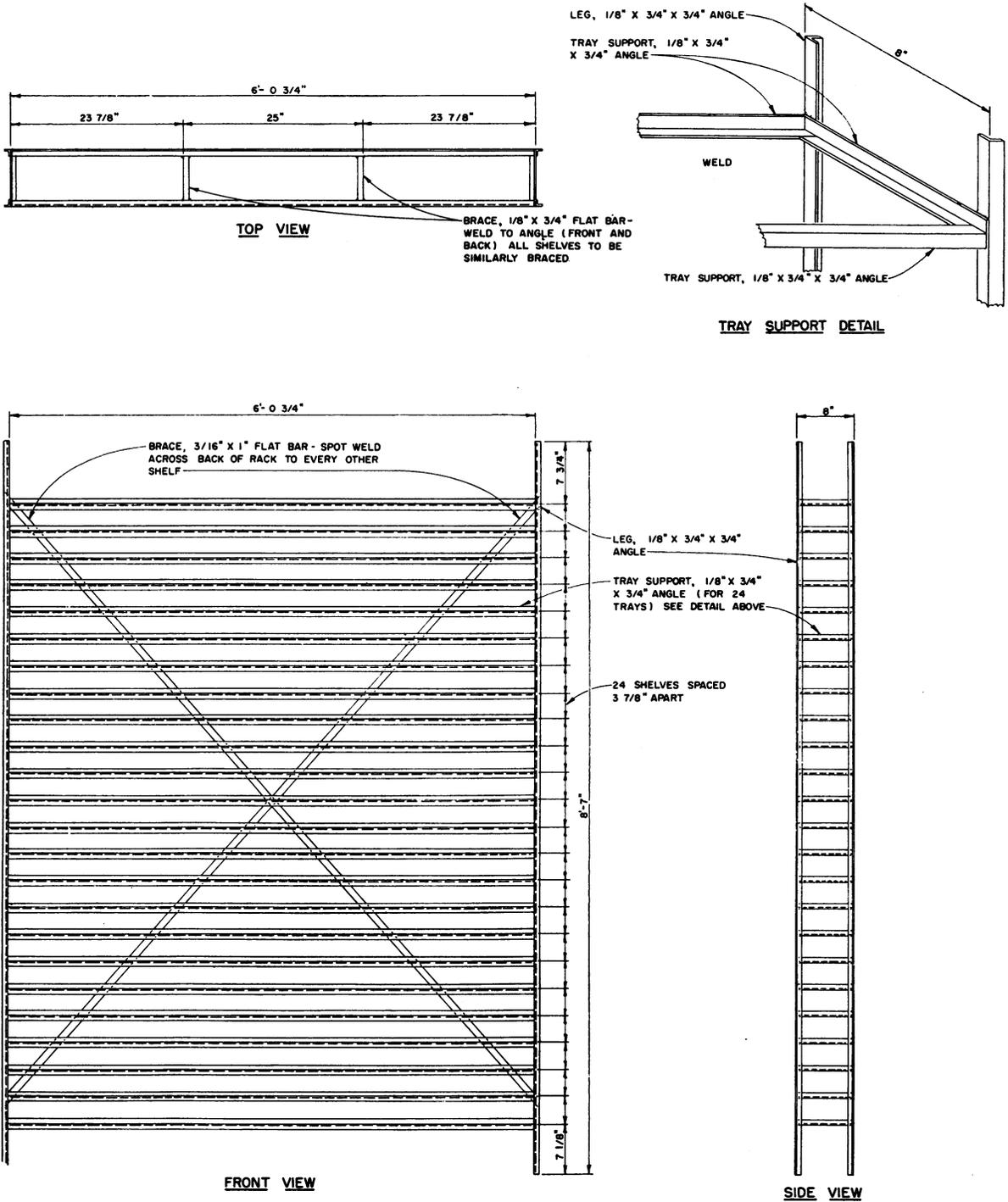
To stock each rearing tray accurately and rapidly, egg strips containing an unknown number of eggs were placed in known amounts of the hatching mixture. Two liters of mixture were satisfactory for up to 20,000 larvae. After 4 hours the egg sheets were removed. The larvae were then dispersed evenly with a two-beater food mixer. Twenty samples, 2 ml. each, of the mixture were taken, using a graduated bacteriological pipette, and the live larvae in each sample were counted. All samples were taken from approximately the same position in the pan—about equidistant between the rotating beaters and the margin of the pan and at half the depth of the mixture. The average number of larvae per milliliter was then calculated and the proper volume was transferred to a rearing container. The method provided estimates of larval numbers that were accurate within  $\pm 5$  percent.

For estimating populations up to 0.5 million larvae in 50-liter mixtures, an agitator-type washing machine was used to disperse the larvae. The machine was modified by cutting

**Figure 2. Larval rearing tray**

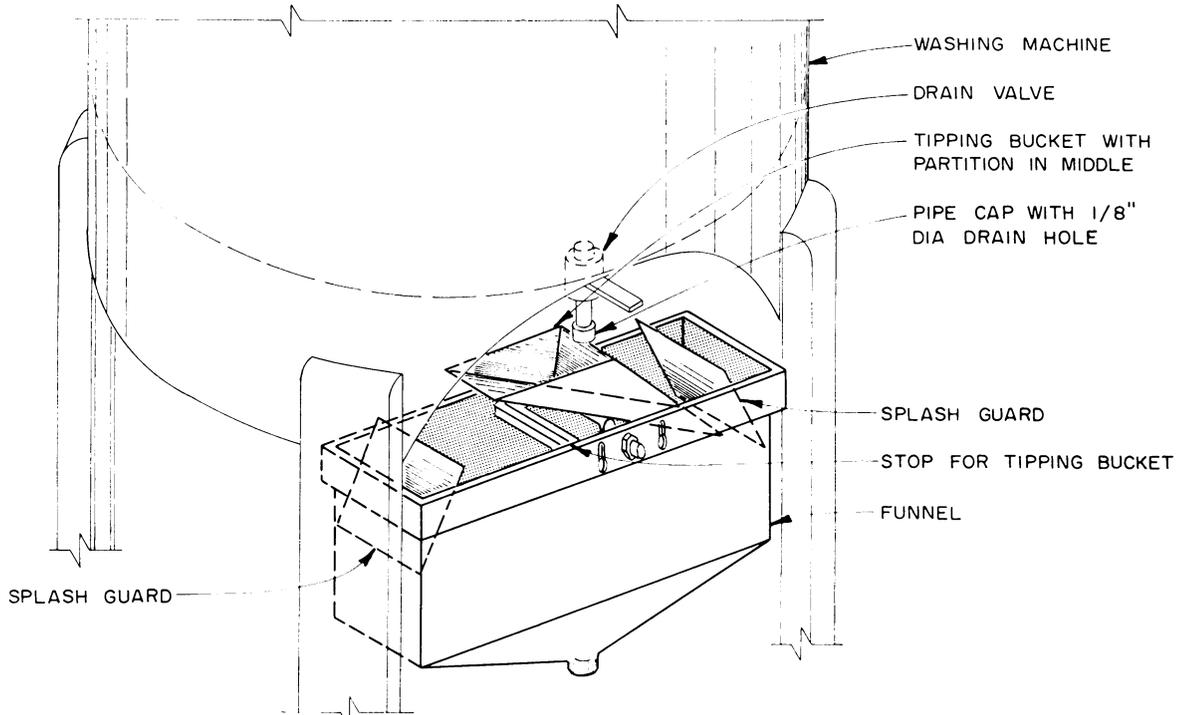


**Figure 3. Racks for larval rearing trays**

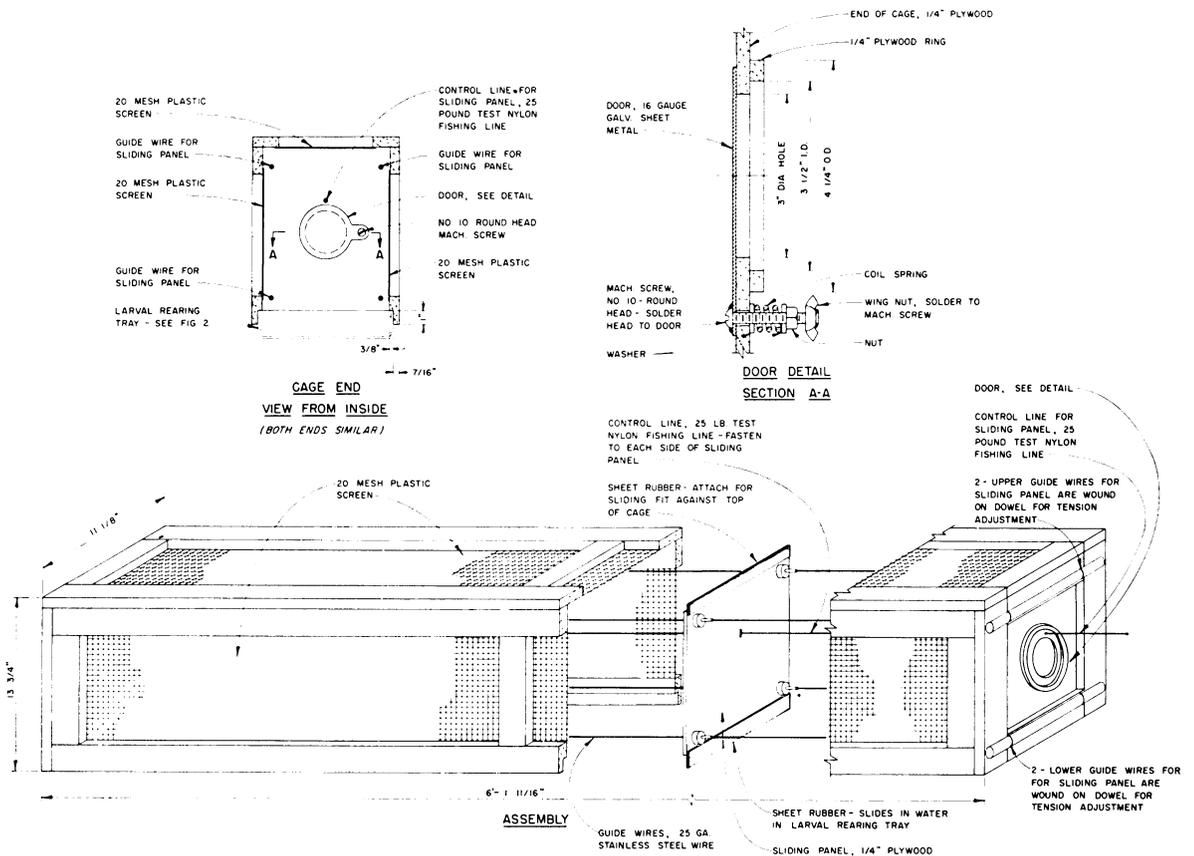


NOTE: RACKS TO BE MADE OF HOT ROLLED STEEL

**Figure 4. Automatic dispenser for obtaining desired volumes of dispersed mosquito larvae**



**Figure 5. Adult mosquito emergence cage**



out sections of the agitator fins to reduce splashing and by installing a plunger-type valve in the bottom of the tub to provide a free-flowing but controllable gravity drain. To permit uniform agitation of small volumes of fluid, three tapered finger-like extensions, about 2 inches long and  $\frac{3}{8}$  inch wide at the base, were attached to the base of the agitator.

After the number of newly hatched larvae dispersed in a known volume of water was calculated, water was added to reduce the density of larvae to 7 per milliliter. One liter of the preparation containing dispersed larvae was then added to 6 liters of water in each rearing tray. Agitation in the machine for periods up to 30 minutes produced no detectable adverse effect on larvae.

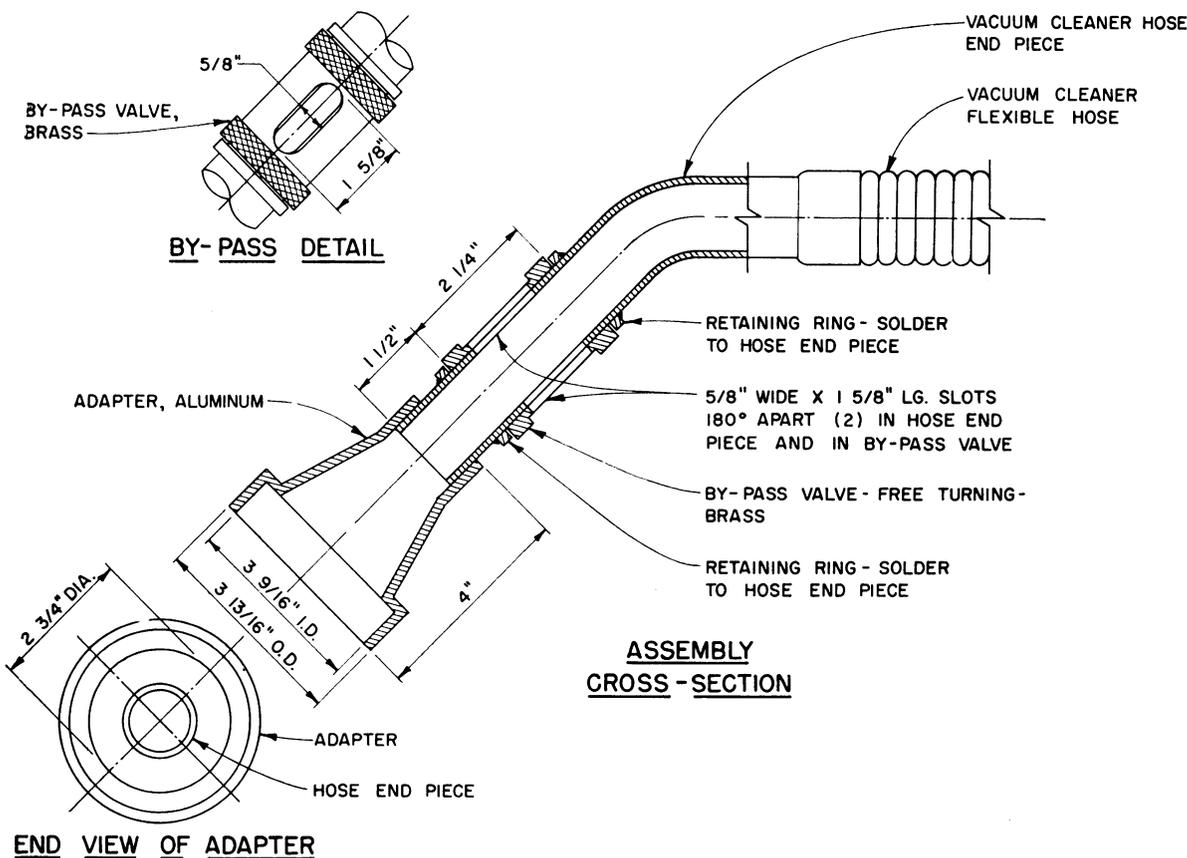
Use of single-liter lots of this preparation for stocking trays was satisfactory for mass rearing; but in stocking experimental trays, greater accuracy was desirable for reliable com-

parative evaluations of culture methods. Differences in numbers of larvae per tray were reduced by dispensing serial aliquots of dispersed larvae. If six experimental trays were to be stocked, each received ten 100-ml. aliquots collected so that the first tray received aliquots 1, 7, 13, 19, 25, 31, 37, 43, 49, and 55; the second tray received aliquots 2, 8, 14, and so forth.

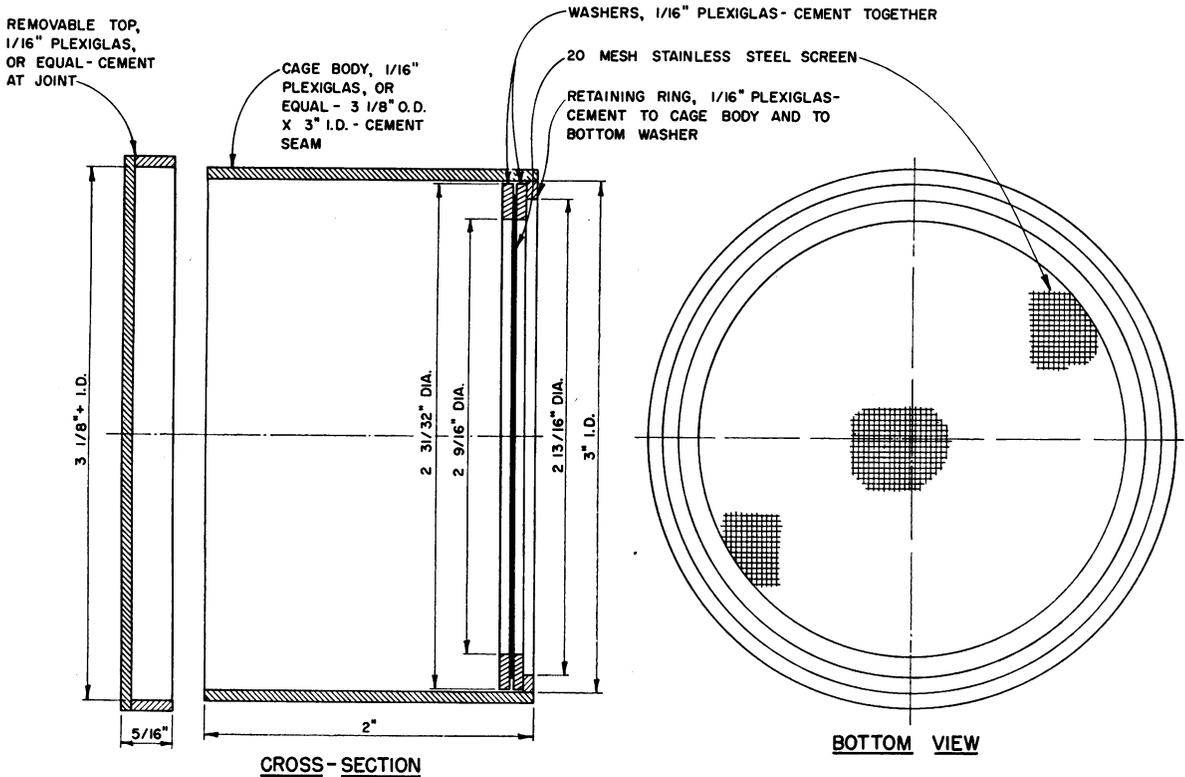
An automatic dispenser was used to obtain the desired volumes of dispersed mosquito larvae. It was patterned after the tripping-bucket rain gauge used by the U.S. Weather Bureau (8). Essentially, it is a pan with two compartments mounted below the valve drain at the bottom of the washing machine tub (fig. 4). As one compartment fills, the weight of the water causes it to tip and empty its contents, and thus the other compartment is presented for filling.

*Larval feeding.* The larvae were fed dog chow (5 percent crude fat) ground to pass

**Figure 6. Vacuum cleaner bypass and adapter used for removing adult mosquitoes from emergence cages**



**Figure 7. Adult mosquito collecting cage**



through a 40-mesh screen. The feeding schedule was 0.2, 0.3, and 0.4 mg. of food per larva on days 0 (day of hatching) through 2, respectively, and 0.6 mg. per larva on days 3 through 7. Plastic spoons of appropriate capacity were constructed and used to measure the scheduled amount of food for the 7,000 larvae in each tray.

*Pupation and emergence.* On the seventh

day after the eggs had hatched, each rearing tray was drained, and the larvae and pupae were removed by straining the medium through a 20-mesh screen. To control accidental and unequal introduction of deleterious scum-forming micro-organisms, rearing trays were cleaned after each use, filled with water, and treated with 0.1 gm. of calcium hypochlorite. After 12 to 24 hours, they were drained and filled with clean untreated water.

**Table 2. Weight in milligrams of unfed *A. aegypti* adults emerging 8-11 days after egg hatch**

Emergence day	Males			Females		
	Number	Total weight	Average weight	Number	Total weight	Average weight
8.....	937	1,313.3	1.40	248	710.6	2.86
9.....	1,049	1,430.3	1.36	751	2,053.4	2.73
10.....	1,160	1,466.5	1.26	1,240	3,226.6	2.60
11.....	481	568.5	1.18	207	498.9	2.41

The larvae and pupae were transferred to trays of fresh water, each accommodating the yield from as many as 17 hatching trays, and an emergence cage (fig. 5) was placed over each tray. The majority of the adults emerged during the following 4-day period. The sliding plywood panel in the emergence cage facilitated rapid collection by concentrating the adults at one end of the cage. A canister-type vacuum cleaner with a bypass and adapter assembly (fig. 6) which produced a vacuum of 2.0 inches manometric water pressure was used to remove the adults through the doors at each end of the emergence cage and concentrate them with-

out injury into plastic collecting cages with screened bottoms (fig. 7). Once in a collecting cage, the adult mosquitoes were readily weighed, anesthetized, or transferred to other containers.

*Adult counting.* Volumetric estimation of adult numbers was too inaccurate for experimental studies, but enumeration by weight proved to be satisfactory. Adults emerging on days 8, 9, 10, and 11 were found to be progressively lighter in weight (table 2). Weight depended upon sex, day of emergence, and larval rearing conditions, so it was necessary to obtain an average weight determination from random samples of males and females on each emergence day. Random samples comprised of approximately 100 males and 100 females were obtained by killing or anesthetizing adults, since it was found that aspirated samples of live adults tended to be biased in favor of males.

## Discussion

With the equipment and techniques described, it was possible for two men to set up 192 trays with a total of 1,344,000 larvae. These men could easily maintain the larvae during their development, concentrate the larvae in the emergence cage trays, remove the adults from the emergence cages, and estimate their numbers. Each brood produced from 0.5 to 0.8 million adults within 11 days of hatching. Limited tests with overlapping of broods indicated that continuous rearing at maximum capacity would produce at least 0.65 million adults every 8 days. Further increases in mosquito production could be obtained by making appropriate increases in equipment and personnel.

This technique was dependable and practical but falls far short of perfection. Continued investigation would undoubtedly result in improved methods. However, in developing the method outlined, numerous modifications were tested and evaluated on the basis of the percentage of larvae that developed to adults within 11 days.

Decreased adult production resulted from (a) daily addition of 70 gm. of sucrose to each larval rearing tray or (b) addition of 0.7 gm. of dog chow to water in rearing trays 12 to 24 hours before adding newly hatched larvae.

No increase in adult production occurred with: (a) sheltering of larval rearing trays from variable light intensities or air movement, (b) daily addition of 15 gm. of sucrose to each larval rearing tray, (c) autoclaving larval feed (250° F. for 15 minutes), (d) transferring larvae from old culture media to fresh media on day 3, or on day 2 and again on day 4.

Limited tests showed an increase in production of adults with the addition of 1 liter of water containing 0.7 gm. of moist yeast cake to each rearing tray on the third day after hatching of larvae. This supplement and a larval density of 10,000 per tray resulted in increased female production, both in terms of percentage of larvae and total numbers. Increasing larval density to 15,000 resulted in increased total numbers of males but decreased numbers of females and reduced the percentages of larvae that became adults in 11 days. As the increased larval density slowed development of females more than males, it provided a means for partial control of sex ratio in each brood of mosquitoes.

## Summary

Equipment and techniques have been developed to :

1. Improve adult colony maintenance.
2. Collect, store, and hatch eggs.
3. Determine the number of larvae, mechanically dispersed, per unit of volume.
4. Dispense desired numbers of larvae automatically.
5. Rear broods of 1.3 million larvae, feeding them ground dog chow on a schedule of 0.2, 0.3, and 0.4 mg. of food per larva on days 0 (day of hatching) through 2, respectively, and 0.6 mg. per larva on days 3 through 7.
6. Concentrate pupae.
7. Collect and estimate numbers of adults from emergence cages.

Each brood produced from 0.5 to 0.8 million adults within 11 days of hatching.

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# Occupational Health Notes

## *Fumes from Fire Extinguisher*

Illness in four persons working in a hardware store in Washington was traced to carbon tetrachloride leaking from a fire extinguisher. A window shade tie-off was attached to the handle of the pyrene extinguisher. The pressure on the handle caused a leak, and the fumes diffused into a small, poorly ventilated office where the employees worked.

## *Pioneer Anti-Noise Law*

A law regulating noise levels inside factories became effective in California on March 15, 1963. A maximum sound-level pressure was established for each octave band. Employers are required to control excessive exposures by isolating noisy equipment, providing workers with ear plugs or ear muffs, or reducing time spent at particularly noisy jobs. The law stems from the increasing number of compensation awards made to California workers for noise-induced hearing loss.

## *Decomposition of Hair Spray*

Heavy carbon monoxide concentrations in a beauty parlor in Michigan were traced to the corrosive action on a heater by the decomposition products of Freon hair spray propellants. Health authorities were called in by a customer who had no-

ticed that several of the employees seemed to be ill. Three of the five beauty operators became so sick that they were unable to work, and the other two had headaches. Air tests showed 200 ppm of carbon monoxide in the working area and 300 ppm in the air being discharged by an overhead heater. The heater was turned off and the building evacuated and aired out. Rust particles from an extremely corroded heat exchanger and vent had plugged the flue, causing all combustion products to return into the building. The corrosive agent was traced directly to the breakdown products of Freon propellant.

## *Ether Spray for Carburetors*

In Indiana, two persons were hospitalized with second and third degree burns received when an automobile carburetor backfired as ether was being sprayed into it to help start the engine. Over-the-counter sale of pressurized cans of ether to be used for starting engines in cold weather has created a fire and explosion hazard to the general public.

## *Restaurant Ventilation*

Ventilation, temperature, and humidity studies in six restaurants in Contra Costa County, Calif., emphasized the advantage of having a ventilation system for the entire dining room and kitchen rather than just a hood over the cooking range. The investigators suggest that warm air from the dining room be used as the makeup air in the kitchen and that the exhaust from a range hood or slot exhaust at the back of the range be discharged directly to the outside air. This arrangement helps control grease in the cooking area, maintains an acceptable temperature in the kitchen, and controls odors in the dining room.

## Program Notes

To increase use of throat culture tests in diagnosing streptococcal sore throat, the Monroe (N.Y.) County Health Department and the county heart chapter have introduced a sterile kit, containing a Dacron throat-culture swab and an insulated bag, for physicians' convenience in mailing specimens to the health department. Findings of positive cultures will be phoned to the physicians, and both positive and negative findings will be mailed to them.

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A program for continuing medical education with breakfast has become popular among general practitioners and specialists in the area of Lake Charles, La., according to an account in the November 1962 *GP*.

The program, begun in 1958, runs from October through April or May for 16 weeks, with meetings in cafeterias or assembly rooms of Lake Charles hospitals on every other Tuesday at 7 a.m. except holiday periods. Following a 15-minute breakfast, the lecture-discussion continues for 30 to 45 minutes; after 8, there is a question-and-answer period. Subjects are selected to fill needs for applicable knowledge. Medical institutions affiliated with Louisiana State University and others in New Orleans provide lecturers. Participants pay no registration fee, and the breakfast is complimentary.

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Because the Brookline (Mass.) Health Department found that few local dentists examined patients for oral cancer and that many dentists had little training in its detection, the department arranged with Tufts University School of Dental Medicine for 20-hour courses for local dentists. The participating dentists, enrolled free, agree to return for refresher sessions and examinations the following 3 years and to serve in the health department's oral cancer

screening and diagnostic clinic. According to Dr. Leon J. Taubehaus, director of the department, who described the program in *Cancer*, (May-June 1962), there is a waiting list for future courses.

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After drinking water from a plastic jug which had contained a cotton defoliant, three members of a family near Josephine, Tex., died of accidental poisoning from arsenic residue. In reporting the incident, the *Texas Health Bulletin* emphasized the risk in re-use of agricultural chemical containers for water or food.

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"Easy on Your Eyes," compiled by the Massachusetts Library Association, lists 600 books in larger-than-normal type. Fulfilling a request from the New England Council of Optometrists, the compilation meets a long-recognized need in libraries. The New England Council of Optometrists, Inc., 101 Tremont Street, Boston 8, Mass., is the publisher.

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In "Fifty Years of Progress," the 1962 annual report of the Des Moines-Polk County (Iowa) Department of Public Health, Dr. James F. Speers, the director, notes that a review of annual reports since 1912 reveals that "most of the successes of which we have been so proud had their basis in efforts made years before, and which were frequently felt to have failed."

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The Muskegon County (Mich.) Health Department, bolstered by a joint APHA and Federal Housing Administration report, a socioeconomic survey of 231 substandard dwellings, and a physical-sanitation report, gained support for better housing standards as a health meas-

ure from nearly every governmental unit and agency in the county. As a result, the welfare department began withholding payments for housing in substandard dwellings. Also a county building inspectors association was formed, leading to widespread adoption of modern building codes.

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The United States Army has begun to test exfoliative cytology as a method for detection of oral cancer. Small samples of surface cells from questionable areas in the mouths of recruits are sent to the Army's Institute of Dental Research for examination.

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The nutrition service of the Iowa State Department of Health has prepared a booklet entitled "Sanitation of Food Service Establishments" to provide on-the-job training of personnel in restaurants, hospitals, nursing homes, custodial homes, schools, hotels, and institutions. The publication is designed for reading by an employee, for excerpting, posting, or for group discussion. Address out-of-State requests to Economics Laboratory, Inc., 250 Park Avenue, New York 17, N.Y.

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Nassau County, N.Y., passed a law in 1962 barring discrimination by hospitals against doctors affiliated with group medical plans, one of the first counties to enact such legislation. In May 1963, a similar law was enacted by New York State.

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In 1952, when New Jersey's population was under 5 million, health departments and local voluntary agencies employed 983 nurses. In January 1962, with a population of more than 6 million and an increased demand for nursing services, only 880 were employed. Governor Richard J. Hughes called a conference on March 8, 1963, to alert citizens to the community nursing need. A limited number of copies of the May 1963 issue of the New Jersey Health Department's *Public Health News* containing the proceedings are available from the New Jersey State Department of Health, Trenton 25, N.J.